The coming of age of the GDNF family and its receptors: gene delivery in a rat Parkinson model may have clinical implications

It took decades before it was realized that NGF (see Ref. 1) had close relatives, and before the dual receptor system of what is now known as the neurotrophin family was discovered. In the case of GDNF (glial cell line-derived neurotrophic factor) the pace of development has been an order of magnitude faster. Thus it was only a few years ago that the discovery of GDNF was published[2,3], yet we already know of one GDNF-related factor called neurturin[4] and of at least two components of a GDNF receptor system, Ret[5,6] and GDNFR-α[7,8]. It is not unlikely that there might be additional GDNF-like proteins, and that Ret, as well as GDNFR-α have relatives. Table 1 illustrates known gene products and guesses at additional possibilities. Clearly, the situation may be less or more complex than outlined depending, for instance, on the specificity between different ligands and receptors as well as on the interactions between the receptor components.

Clinical hopes and concerns

As for all newly discovered trophic factors, there is hope that GDNF might become useful in the treatment of nervous system disease. Given its spectrum of demonstrated activities which includes, but is not limited to, potent trophic actions on dopamine neurons, motoneurons and peripheral ganglia[2,9–12], these hopes include treatments for Parkinson's disease, ALS, and peripheral neuropathies. Perhaps the best-studied experimental model to date in which GDNF has proven effective is that of lesioning the dopamine neurons of the ventral midbrain (substantia nigra, and the ventral tegmental area) to model Parkinson's disease. There is now ample evidence in both rodents and primates that delivery of GDNF protein to the CNS can not only counteract lesions and neurotoxic assaults on the dopamine neurons, but also stimulate dopamine neurons to perform better (see Ref. 13).

Most molecules with potent neurotrophic effects are proteins. Therefore, in the case of the brain, oral administration is precluded because of the digestive enzymes of the gastrointestinal tract and intravenous administration because of the blood–brain barrier. The situation may be different for peripheral neuropathies or ALS (assuming that proteins can reach motoneurons via the neuromuscular endplate); trials using subcutaneous delivery routes are under way. While pilot studies delivering NGF directly intracerebrally[14,15] or into the CSF[16] using pumps and indwelling catheters have been carried out, better systems for long-term and/or localized delivery are clearly needed.

How to increase the level of a trophic factor in the brain

In addition to the direct stereotaxic protein injection approach, there are many additional ways in which the goal of controlling the level of a trophic factor in the CNS might be achieved (see Ref. 16). One set of techniques utilizes the protein itself: implantable, biodegradable, slow-release preparations containing the protein can be used to obtain long-term localized release. Another approach is to cross the blood–brain barrier, for instance by coupling the active protein to a molecule which is normally translocated across the barrier from the blood stream[17]. A third approach is to find low molecular weight trophic factor receptor agonists that can pass the blood–brain barrier. While this is an attractive approach, it has proven notoriously difficult to find good low molecular weight agonists (and antagonists) for peptide receptors. A fourth approach to the problem is based on an understanding of mechanisms that regulate endogenous production of the factor at hand. Thus, it may become possible to pharmacologically stimulate endogenous synthesis of a given trophic factor. A fifth principal approach is to transfer genes to the brain to produce the desired factor. This in turn can be achieved in different ways, such as grafting cells that normally make the needed factor or cells that have been genetically engineered to synthesize large amounts of a factor. Perhaps the most promising future method for trophic protein delivery to the CNS is to transfer the required genes not by using cells, but using much smaller carriers such as those offered by certain modified viruses capable of functionally incorporating desired genes into mammalian cells.

Intracerebral GDNF gene delivery rescues neurons

Recently, Choi-Lundberg and colleagues[18] reported that a replication-deficient adenoviral vector carrying the human GDNF gene, when injected into the mesencephalon of rats, could protect dopamine neurons from undergoing 6-OHDA-induced degeneration. In these experiments the authors first labeled dopamine neurons by retrograde filling with fluorogold injected into the terminal areas in striatum. One week later, the DA neurotoxin 6-OHDA was injected into one striatum[19] and the ensuing loss of

TABLE 1. The GDNF family and its receptors

Factors	Receptor components	
	Binding component(s)	Transducing component(s)
GDNF	GDNFR-α	c-Ret
Neurturin	(GDNFR-β?)	(Ret-β?)
(GDNF-3?)	(GDNFR-γ?)	?
(GDNF4?)	?	?

The four known gene products for factors and receptors are tabulated. Terms within brackets and with question marks are hypothetical and indicate that the field is developing rapidly.

Lars Olson
Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

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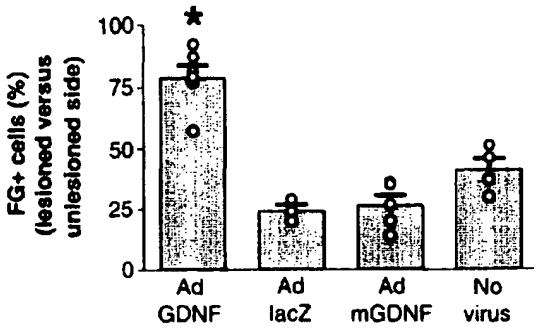

Fig. 1. *Results of counting DA neurons in the experiments carried out by Choi-Lundberg* et al.[18] *Dopamine neurons were prelabeled by retrograde transport of fluorogold (FG) injected into striatum on both sides. At the same time, the adenoviral GDNF construct was injected over the substantia nigra on one side. Control animals were injected with a mutant non-functional form of GNDF (mGDNF), or a lacZ marker gene construct. One week later the number of FG-positive DA neurons was counted. The number of surviving DA neurons in the Ad GDNF is significantly higher than in all three controls. From Choi-Lundberg* et al.[18]

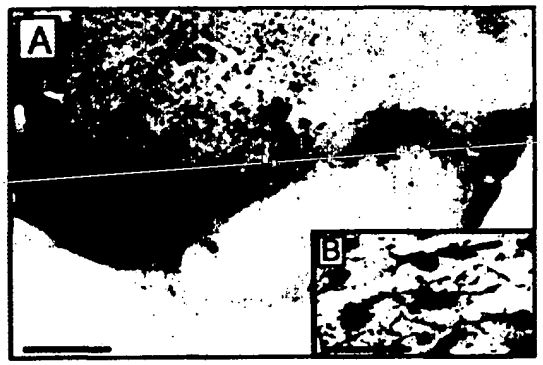

Fig. 2. *Adenoviral constructs can infect DA neurons and lead to transgene expression* **(A)**. *In this control experiment, an adenoviral* lacZ *construct was injected above the substantia nigra area. lacZ with a nuclear localizing signal leads to the presence of β-galactosidase in the nucleus of many cells (blue stain), above and partly within the area of the DA neurons of substantia nigra and the ventral tegmental area. The section was double-stained with an antibody to tyrosine hydroxylase (TH) specifically to localize DA neurons (brown stain). As illustrated in the higher power in the inset* **(B)**, *there are TH-positive, β-galactosidase-positive, as well as one example of a TH–β-galactosidase double-stained neuron, proving that the DA neurons themselves can be infected and express a transgene. From Choi-Lundberg* et al.[18]

DA cell bodies was followed by cell counting methods four weeks later. Choi-Lundberg *et al.* found that an injection of the adenoviral GDNF gene construct immediately over the DA cell bodies one week prior to the 6-OHDA challenge rescued a large proportion of the nerve cell bodies destined to die, and that a control construct carrying a mutant, nonfunctional GDNF gene had no such rescuing effect.

How long will transg n s stay active?

Choi-Lundberg and colleagues measured levels of DNA, RNA and protein in mesencephalon following injections of the viral constructs and found significant amounts after one week. While GDNF protein was still detectable after four weeks by ELISA, levels of both protein and RNA (but not DNA) had dropped to about a third of the levels noted at one week. The authors therefore suggest that there had been a down-regulation of the Rous sarcoma virus promoter used to drive the expression, rather than a loss of infected brain cells. They discuss the declining transgene expression, noting that others have had the same problem (see Ref. 19), but remain optimistic that future versions of the transgene approach may overcome the problems of down-regulation.

It is known that a single injection of GDNF can have long-lasting effects (a week or more). Slow-release preparations could be expected to extend this period to months rather than weeks. As the gene delivery technique now stands, it therefore offers limited advantages over the direct protein-delivery methods. It may also be associated with specific disadvantages relating to the addition of more foreign proteins (see Ref. 20). Choi-Lundberg *et al.* described tissue reactions caused by the transgene delivery and found that 'mild' and 'moderate' cellular reactions occurred following both GDNF and mutant GDNF transgene delivery, possibly representing immune responses or other inflammatory reactions. Novel virus-based constructs, based e.g. on the adenoassociated virus (AAV) may overcome some of these problems, since much fewer viral genes need to be expresed. AAV itself has also been declared a non-pathogen for humans.

Can transgene expression be directed and controlled?

In the reported experiments[18], infected cells probably included some of the DA neurons themselves, since control animals infected with *lacZ* and a nuclear-localizing signal had DA neurons that could be double-labeled for β-galactosidase (the product of the *lacZ* gene) in the nucleus, and tyrosine hydroxylase (a marker of DA neurons) in the cytoplasm. It is not known if DA neurons can utilize self-produced GDNF, but following release autocrine or paracrine effects appear likely, given the presence of both Ret and GDNFR-α in DA neurons (Figs 1–3). It might also become possible to direct expression to certain cell types by including the appropriate promoters. Finally, inclusion of other regulatory elements may allow temporal pharmacological control of transgene expression levels.

The long and winding road to possible clinical applications

Trophic factors are characteristically very potent and always have a rather wide spectrum of effects. Therefore delivery of

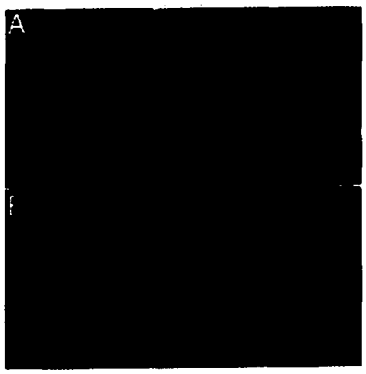

Fig. 3. *Dopamine neurons have the dual GDNF receptor system.* In situ *hybridization showing the presence of GDNFR-α mRNA in the SN and VTA areas of a newborn rat is shown in* **(A)**. **(B)** *illustrates the presence of ret mRNA in dopamine neurons of an adult rat. Modified from Nosrat* et al.[22]

trophic factors in a highly localized fashion may be necessary to avoid unwanted effects. For instance, when NGF was delivered directly into putamen of patients with Parkinson's disease (to support grafts of adrenal medullary tissue) there were no noticeable side effects[15]. However, when NGF was delivered to the CSF of patients with Alzheimer's disease (to stimulate cholinergic neurons) there were two negative effects[16] (loss of appetite and pain associated with movements) which might be related to the widespread distribution of the factor in and from the cerebrospinal fluid compartment. Such negative effects may be related to the transient Schwann cell hyperplasia and sensory and sympathetic sprouting in subpial compartments of the spinal cord that have been described in rats following cerebroventricular delivery of NGF[21].

Finally, it is being increasingly realized that a given type of neuron needs a combination of factors, rather than a single trophic factor, for optimal support and function. Therefore, in a given disease state, it might not suffice with one factor, even though that particular factor has proven to be trophic for the neurons at hand and has worked in animal experiments. The human disease may also differ significantly from the animal model. Early disappointments with clinical trials of new drugs are commonplace and should only challenge us to improve our protocols. In this respect, the study of Choi-Lundberg and colleagues is an important contribution, demonstrating feasibility of the viral delivery strategy for GDNF in a model of a common neurodegenerative disease.

References

1 Levi-Montalcini, R. *et al.* (1996) *Trends Neurosci.* 19, 514–520
2 Lin, L. *et al.* (1993) *Science* 260, 1130–1132